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# Cognitive and social functions and growth factors in a mouse model of Rett syndrome

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ABSTRACT

Rett syndrome (RTT) is an autism-spectrum disorder caused by mutations in the X-linked gene encoding methyl-CpG-binding protein 2 (MeCP2). Abnormalities in social behavior, stereotyped movements, and restricted interests are common features in both RTT and classic autism. While mouse models of both RTT and autism exist, social behaviors have not been explored extensively in mouse models of RTT. Here, we report cognitive and social abnormalities in *Mecp2*$^{1lox}$ null mice, an animal model of RTT. The null mice show severe deficits in short- and long-term object recognition memories, reminiscent of the severe cognitive deficits seen in RTT girls. Social behavior, however, is abnormal in that the null mice spend more time in contact with stranger mice than do wildtype controls. These findings are consistent with reports of increased reciprocal social interaction in RTT girls relative to classic autism. We also report here that the levels of the neurotrophins brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1), and nerve growth factor (NGF) are decreased in the hippocampus of the null mice, and discuss how this may provide an underlying mechanism for both the cognitive deficits and the increased motivation for social contact observed in the *Mecp2*$^{1lox}$ null mice. These studies support a differential etiology between RTT and autism, particularly with respect to sociability deficits.



## 1. Introduction

Rett syndrome (RTT), an autism spectrum disorder observed primarily in females, has a phenotype that includes reduced head growth, regression of motor skills, and severe social and cognitive deficits, as well as abnormalities in neuroanatomy, metabolism, and biochemistry [1–4]. RTT is the only autism-spectrum disorder with a known genetic cause. More than 90% of RTT cases are associated with mutations in the X-linked gene encoding methyl-CpG-binding protein 2 (MeCP2), a transcriptional repressor that binds methylated DNA [5]. RTT girls, who are generally heterozygous with one normal and one mutated *MECP2* gene, have varying phenotypes due to random X-inactivation patterns and variable *MECP2* mutations. Males who are null for MeCP2 expression are more severely affected and often do not survive birth. These phenotypic and neuroanatomical/biochemical features of RTT are also observed in several *Mecp2* mouse models of RTT, and are particularly well replicated in the males null for Mecp2 [6–8].

Although a number of characteristics clinically differentiate RTT from classic autism, RTT is currently classified as an autism-spectrum disorder. Because RTT superficially reflects several characteristics of autism, many researchers have proposed RTT as a model for understanding autism. Both RTT and autistic children are resistant to learning new things, have restricted interests, giggle without evident cause, are socially isolated, and exhibit stereotypic playing habits. However, several important clinical distinctions exist between the two disorders. RTT girls are more likely than autistic children to initiate social interactions, make eye contact and respond actively to music [9]. Furthermore, RTT girls frequently exhibit abnormalities that are not common in either autism or in severe mental retardation without autistic features, including significant regression of speech and motor skills, deceleration of head growth, scoliosis, and respiratory abnormalities [10]. Although autism and RTT share several symptoms, the differences in phenotypic features, particularly related to social interactions, respiratory abnormalities, and the regressive nature of RTT have lead some researchers to question the classification of RTT as an autism-spectrum disorder. Because of the clinical differences between RTT and autism, it is critical that we pay careful attention to those characteristics that could potentially distinguish the two syndromes when we evaluate animal models as they may provide insight into the underlying mechanisms of these similar but perhaps distinct neurodevelopmental disorders.

In mouse models of RTT, motor and respiratory deficits are clear and profound, recapitulating those deficits noted in girls with RTT (see [11] for a recent review). Alterations in cognitive and social behaviors, however, have been less well studied but generally suggest mild to severe impairments that are consistent with reports in RTT girls [11]. Cognitive deficits in fear conditioning, spatial learning and memory, and object recognition have been noted in several mouse models of RTT with impairments ranging from severe to mild: in null

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*Mecp2*$^{1lox}$ mutants, deficits are relatively severe with an onset by 6 weeks of age [8]; in mutants with conditional knockout of Mecp2 in forebrain structures, deficits are moderate [12]; and in *Mecp2*$^{308/Y}$ mice, who express a truncated form of the Mecp2 protein, deficits are relatively mild with an onset at 20+ weeks of age [13]. A few reports document abnormal social behavior in *Mecp2* mutant mice. Home cage social behavior is altered in *Mecp2*$^{308/y}$ mice; mutants spend significantly less time building nests and have more poorly constructed nests. It is important to note that decreased nest building may be indicative of decreased sociability but may also indicate reduced forelimb coordination in the mutants [14]. Both *Mecp2*$^{308/y}$ and conditional *Mecp2* knockout mice exhibit reduced social approach behaviors [12,14], whereas decreased expression of Mecp2 in a subset of neurons in the amygdala in mice results in reduced juvenile play but normal social approach behaviors [15]. Mice lacking Mecp2 in Sim-1 expressing neurons in the hypothalamus approach same mice species similarly to controls but are significantly more aggressive than controls [16]. To our knowledge, social behaviors have not been characterized in *Mecp2* null mice.

The reported deficits in motoric and respiratory functions in mouse models of RTT complicate the assessment of cognitive and social deficits, because most cognitive and social tasks in mice require significant movement and respiratory function for normal performance. Undoubtedly, this issue is also a challenge for clinicians assessing these behaviors in RTT girls. In order to address this issue in the current study, we have chosen carefully behavioral tasks that have relatively modest demands for motility. We assess cognitive performance using an object recognition task, which relies on close contact with objects rather than swimming or running behaviors, and assess social behavior with social approach and social novelty tasks. In both cases, the apparatuses in which behavior is measured have been adapted from standard dimensions [8,17,18] to reduce the motoric demands of the tasks.

The level of expression of several growth factors is linked to performance on cognitive and social tasks. Therefore, in a second set of experiments, we examine growth factor levels in several cortical brain regions implicated in cognitive and social behaviors. Growth factors promote neurite outgrowth, neuronal differentiation, and maintenance of cell function and survival [19]. Three extensively studied growth factors are insulin-like growth factor 1 (IGF-1), brain-derived growth factor (BDNF), and nerve growth factor (NGF). The latter two are neurotrophins, a class of growth factors that play a significant role in postnatal survival and neurite outgrowth. While all three growth factors have different expression profiles and act on different classes of neurons, they all promote neuronal integrity. In the mouse brain, IGF-1 is associated with overall brain size [20,21] and is widely expressed in fetal brain tissue, with more discrete postnatal expression in olfactory bulb, hippocampus, and cerebellum [22]. BDNF and NGF expressions are particularly high in the hippocampus, cerebellum, cortex, striatum and basal forebrain and exert trophic effects in those regions [23–26].

Both during development and in adulthood, IGF-1, BDNF and NGF support activity-dependent neuronal plasticity underlying cognitive processes such as learning and memory [27–31]. A wide variety of studies shows that reduced neurotrophin levels are associated with reduced neuronal complexity and impaired learning, and many of these effects can be restored with increased neurotrophin levels. More recently, a number of studies highlight a significant role for neurotrophins in complex social interactions. Although the studies are not always consistent, higher levels of neurotrophins are generally associated with more aggression and reduced social interactions, while decreased neurotrophin levels are associated with socially submissive behaviors and increased social bonding [32,33]. This relationship between neurotrophins and social behavior has been documented in rodents and non-human primates, as well as humans [34–39].

Interest in neurotrophins and RTT has been sparked by reports that BDNF is a target of MeCP2 regulation [40] and IGF-1 reverses some motor and respiratory deficits in *Mecp2* null mice [41]. However, there is conflicting evidence about growth factor levels in RTT. RTT girls have unchanged levels of cerebrospinal and serum IGF-1 levels compared to controls [42,43], and IGF-1 levels appear normal in the striatum and cerebellum of *Mecp2* null mice [44]. There are conflicting reports about NGF levels in RTT. NGF is either reduced [42,45–47] or unaltered compared to control subjects [48,49], with the results being dependent on age, pathology, and sample collection site. We have found that NGF levels are unaltered in striatum or cerebellum in *Mecp2* null mice [44], however, NGF levels in other brain regions have not been reported. BDNF levels are low in RTT [50], which has been confirmed in several mouse models of the syndrome [44,51,52].

The focus of the current study is on social and cognitive behaviors in *Mecp2* null mice, as well as neurotrophic changes that may support these behaviors. In this study, we show that null *Mecp2*$^{1lox}$ mice are impaired in both short and long-term memories on an object recognition task. However, the mutants are not impaired on a social approach or social novelty task; rather the mutants stay in closer contact with a stranger mouse and show more interest in a new stranger than do controls. IGF-1, BDNF and NGF expression levels are all reduced in the hippocampus but not in prefrontal or somatosensory cortical areas; these changes in neurotrophins may underlie the reduced cognition, enhanced sociability in the *Mecp2*$^{1lox}$ null mice.

## 2. Materials and methods

### 2.1. Experimental animals

All experiments were conducted on *Mecp2*$^{1lox}$ null mice [6] and their C57BL/6 male wildtype (WT) littermates, with procedures approved by the Wellesley College Institutional Animal Care and Use Committee, conforming to the standards set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. *Mecp2*$^{1lox}$ female heterozygous founder mice (a gift from Dr. R. Jaenisch) were back-crossed with C57BL/6 males to establish and maintain a colony of *Mecp2*$^{1lox}$ mutants. Offspring were weaned on postnatal day (PD) 22, housed in cages with up to 5 same sex littermates, and maintained on a 12 h light/dark cycle with lights on at 0700 and food and water provided *ad libitum*. The same mice were used for both the object recognition and social approach behavioral tasks. Pups were genotyped as described previously [53].

### 2.2. Behavioral assays

#### 2.2.1. Object recognition on PD 29–30

Non-spatial object memory was assessed during three sessions adapted from previously published methods [54]. This task relies on the innate desire of a mouse to explore unfamiliar objects versus familiar objects. Testing was performed in an open-field arena (42 cm × 58 cm × 21 cm) constructed of clear plastic. Three stimulus objects were used that varied in shape, color, and texture, but were similar in size (around 8 cm × 8 cm × 8 cm).

On the day prior to testing, mice (WT: $n = 10$; null: $n = 7$) were habituated to the empty testing apparatus for 5 min. During the training session on PD 29, two identical copies of Object 1 were positioned 5 cm from the side wall and equidistant from the front and back walls. The mouse was placed in the center of the arena and allowed to explore both objects for 10 min. Mice were tested for short-term object memory between 90 and 120 min after the completion of the training session. There was no significant difference in performance between 90 (WT $n = 9$; null $n = 4$) and 120 (WT $n = 1$; null $n = 3$) minutes post-training for WT and null mice, so data for each genotype were pooled. The mouse was returned to the arena that contained both the now familiar Object 1 and a novel object,